Protocol – Parafin embedding 3D cultures for sectioning and histology techniques

Notes: This protocol refers to 3D Matrigel cultures established in 96 well plates. This protocol may need to be adapted for other size cultures or other types of 3D cultures.

Methods

1. Fix 3D cultures in 10 % neutral formalin buffer (NBF) overnight.
2. Prepare 10 ml working solution of 4% agarose melted in dH_20. Keep the agarose melted in a heating block.
3. Place the microscope slide flat on a bed of ice.
4. Remove 3D cultures from 96 well plates and transfer to an Eppendorf tube. To facilitate removal of 3D cell culture sample from well, first scrape bottom of plate with blunt forceps (pinched together). Pipette the sample using p200 pipette tip with the end snipped off to increase opening.
5. Microcentrifuge sample at maximum speed for 10 secs to pellet. Remove NBF with pipet. Add ~100 ul PBS to each sample.
6. Pipette to loosen pellet. Place on glass slide. Pipette 100-300 ul melted agarose onto pellet so that the sample is completely covered. Cut away excess agarose using razor blade or knife.
7. Process samples for paraffin embedding by incubating in:
    a. 75% ethanol for minimum of 1 hour at room temperature
    b. 95 ethanol for minimum of 1 hour at room temperature
    c. 100% enthanol for minimum of 1 hour at room temperature
    d. Isopropanol for minimum of 1 hour at room temperature
    e. Isopropanol:paraffin 50:50 for 2 hours or more at 60^{o}c using oven.
    f. Paraffin 100% for 24- 48 hours at 60^{o}c
8. Fasten to paraffin cassette using a small mold. Make sure there is enough wax at the base of the cassette.
9. Remove sample from oven and let sit on counter for at least 2 hours to resolidify.
10. Place in the refrigerator for at least 1 hour to facilitate removal of sample from mold.
11. Section for histology. Recommend 5-7 micron sections.
12. Process for histology as you would for tissue sections using xylenes and ethanols. Check dewaxed slides under the microscope for cells. Circle your sample with a hydrophobic marker or sharpie so you know where your sample is located on the slide.


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